ORIGINAL ARTICLE

Asian Herpetological Research 2020, 11(2): 95–107
DOI: 10.16373/j.cnki.ahr.190068

A New Species of the Genus *Rhabdophis* Fitzinger, 1843 (Squamata: Colubridae) in Southwestern Sichuan, China

Yige PIAO[1,2#], Zening CHEN[1,3#], Yanqing WU[4], Shengchao SHI[1,3], Hirohiko TAKEUCHI[5], Teppei JONO[6], Masaya FUKUDA[7], Akira MORI[7], Yezhong TANG[1], Qin CHEN[1*] and Li DING[1*]

[1] *Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, Sichuan, China*
[2] *University of Chinese Academy of Sciences, Beijing 100049, China*
[3] *Key Laboratory of Bio-Resource and Eco-Environment of Ministry of Education, College of Life Sciences, Sichuan University, Chengdu 610065, Sichuan, China*
[4] *Nanjing Institute of Environmental Sciences, Ministry of Ecology and Environment of China, Nanjing 210042, Jiangsu, China*
[5] *College of Bioresource Sciences, Nihon University, Fujisawa, Kanagawa 2520880, Japan*
[6] *Tropical Biosphere Research Center, University of the Ryukyus, Nishihara, Okinawa 9030213, Japan*
[7] *Department of Zoology, Graduate School of Science, Kyoto University, Sakyo, Kyoto 6068502, Japan*

Abstract The genus *Rhabdophis* is a group of widely distributed snakes with more than 20 species. Recent field surveys uncovered a species in southwestern China, which has long been considered as *R. pentasupralabialis*. Combined molecular and morphological analyses revealed it as a new species *Rhabdophis chiwen* **sp. nov.** Based on 12 specimens, this new species is distinguished by the following characters: 1) dorsal body saddlebrown, dorsal scales typically with black margins forming spots and stripes, the margin of the outer row forming two faint dorsolateral black cross-bars alongside body; 2) ventral scales 151–159, the outer margin of ventral scales and several lateral rows of dorsal scales forming ventrolateral longitudinal brownish-red coloration, with faint black spots in the middle of ventral scales; 3) a black oblique stripe present below eyes, often with a black spot between the 2nd and 3rd supralabial and a black stripe on the 5th supralabial; 4) eyes dark khaki, pupils black; 5) infralabials usually 7, the first four in contact with anterior chin-shields; 6) temporal scales 1+1; 7) dorsal scales in 15 rows, feebly keeled except the outer 1–2 rows; 8) anal scale divided; subcaudals 45–59; 9) preocular 1 and postoculars 3 (occasionally 2); 10) body medium-sized (snout-vent length: adult males 404–431 mm, adult females 409–476 mm); 11) tail moderate (tail length/total length in adult males 0.205–0.238, in adult females 0.172–0.193). With the discovery of this new species, the total number of species in genus *Rhabdophis* is 28 with 12th species known to occur in China.

Keywords *Rhabdophis chiwen* **sp. nov.**, morphology, Natricinae, phylogenetics, taxonomy

1. Introduction

The genus *Rhabdophis* is a group of widely distributed snakes with a unique defensive system that relies on nuchal glands (Mori *et al.*, 2012). In 1960, Malnate defined this genus based on morphological characters, i.e. hemipenes with sulcus spermaticus divided; last two maxillary teeth strongly enlarged, recurved and usually preceded by a diastema; internasals broad anteriorly, nostrils lateral; apical pits present or absent; vertebral glands present in several species (Malnate, 1960).

Currently, 27 species are recognized and are distributed in eastern and southern Asia (Zhu *et al.*, 2014; Takeuchi *et al.*, 2018). Two new species were discovered in the past five years, *Rhabdophis akraios* (Doria *et al.*, 2013) and *Rhabdophis guangdongensis* (Zhu *et al.*, 2014).

#Both authors contribute equally to this work.
*Corresponding authors: Dr. Li DING, from Chengdu Institute of Biology (CIB), Chinese Academy of Sciences (CAS), with his research focusing on biogeography, systematics, and taxonomy of reptiles (snakes); Dr. Qin CHEN, from CIB, CAS, China, with his research focusing on behaviour and electrophysiology of infrared organs of Crotalidae, acoustic communication of Anura.
E-mail: dingli@cib.ac.cn (Ding L.); chenqin@cib.ac.cn (Chen Q.)
Received: 19 December 2019 Accepted: 20 April 2020

Eleven species of *Rhabdophis* are now known to occur in China (Zhu *et al.*, 2014), which are *R. adleri* ZHAO, 1997, *R. guangdongensis* Zhu, Wang, Takeuchi and Zhao, 2014, *R. himalayanus* Günther, 1864, *R. lateralis* Berthold, 1859, *R. leonardi* Wall, 1923, *R. nigrocinctus* Blyth, 1855, *R. nuchalis* Boulenger, 1891, *R. pentasupralabialis* Jiang and Zhao, 1983, *R. subminiatus* Schlegel, 1837, *R. formosanus* Maki, 1931 and *R. swinhonis* Günther, 1868. During several field work in the southwestern part of Sichuan Province, China, we collected a number of samples which were originally referred to as *R. pentasupralabialis*. Nevertheless, those snakes can be distinguished from *R. pentasupralabialis* based on morphological characters. Moreover, the phylogenetic analysis based on the mitochondrial cytochrome *b* (*cyt b*) and nuclear oocyte maturation factor (*cmos*) gene sequences revealed that those samples are different from *R. pentasupralabialis* and other congeners in China. Therefore, we propose that those specimens be a new species in the genus *Rhabdophis*.

2. Materials and Methods

2.1. Specimens sampling Specimens were collected from the Xingou Village (XG, 10 specimens) in Tianquan County and Jiguan Mountain (JG, 2 specimens) in Chongzhou City, Sichuan Province, China (Table 1). Fresh liver or muscle tissues were taken and immediately preserved in 95% ethanol and stored at –20 °C. The specimens were dehydrated in absolute ethanol and then later transferred to and stored in 70% ethanol. In addition, another 10 samples of *R. leonardi* (4 specimens), *R. nuchalis* (4 specimens) and *R. pentasupralabialis* (2 specimens) that represent 3 sympatric species with relatively close niche in Sichuan Province, were used for molecular analyses.

2.2. Molecular phylogenetic analysis Genomic DNA was extracted from the collected tissue samples in Chengdu Institute of Biology (CIB). PCR amplifications were performed in 25 μl reactions (12.5 μl I-5™ 2×High-Fidelity Master Mix, 10 μl ddH_2O, 1 μl F-primers, 1 μl R-primers, 0.5 μl DNA template) using the following cycling conditions: initial denaturation for 2 min at 95°C, followed by 35 cycles: denaturation at 94 °C for 40 s, annealing at different temperatures (48.5 °C for *cyt b* or 56 °C for *cmos*) for 25 s, elongation at 72 °C for 15 s, and then finalized with elongation step of 2 min at 72 °C, with the PTC-100 thermal cycler (BioRad, USA). Purified PCR products were sequenced using the same PCR primers. Sequencing was completed by Beijing Qingke New Industry Biotechnology Co., Ltd. Sequences for comparison of available species were downloaded from GenBank. *Amphiesma stolatum* and *Natrix natrix* were used as outgroups.

The *cyt b* and *cmos* sequences were combined into one dataset to build gene trees. All sequences were aligned with other retrieved sequences in the same gene loci by using software MEGA 7 (Kumar *et al.*, 2016). Raw trace files were edited in Geneious 7 (Biomatters Limited, New Zealand). Partition finder 2.1.1 under BIC identified the optimal models of sequence evolution for each partition (Lanfear *et al.*, 2012). Phylogenetic relationships derived from the combined gene fragments were performed based on Bayesian Inference (BI) by using MrBayes 3.2 (Ronquist *et al.*, 2012). We ran our analysis for 20 million generations with the chains, sampling every 1000 generations. The average standard deviation of split frequencies (ASDSF < 0.01). The first 1000 trees (of 20,000) were discarded as the burn-in. A 50 majority-rule with compatible groups consensus was taken from the remaining trees and posterior probabilities (pp) of 0.95 or above were considered significant. We also performed maximum-likelihood (ML) analysis by using the program RaxML v8 (Stamatakis, 2014) and IQ tree (Nguyen *et al.*, 2015). Reliability of the ML tree was assessed by calculating bootstrap probability (BP) with 1000 replications. Additionally,

Table 1 Morphological characters of *Rhabdophis chiwen* **sp. nov.**

	No.	SVL	Tal	SPO	PRO	PTO	LR	TEM	SL	IL	VEN	DSR	SC	Sex	Locality
Holotype	CIB116092	422	118	1	1	L:2 R:3	1	1+1	5	L:7 R:6	152	15-15-15	56	Male	JG
Paratype	CIB116093	431	131	1	1	3	1	1+1	5	7	155	15-15-15	57	Male	JG
Paratype	CIB116094	411	113	1	1	3	1	1+1	5	7	154	15-15-15	55	Male	XG
Paratype	CIB116095	461	96	1	1	L:3 R:2	1	1+1	5	L:6 R:7	157	15-15-15	45	Female	XG
	CIBDL1807132	427	128	1	1	3	1	1+1	5	L:8 R:7	154	15-15-15	58	Male	XG
	CIBDL1807133	409	128	1	1	3	1	1+1	5	7	157	15-15-15	58	Male	XG
	CIBDL1807134	411	98	1	1	L:3 R:2	1	1+1	5	L:7 R:6	159	15-15-15	49	Female	XG
	CIBDL1807135	476	107	1	1	3	1	1+1	5	7	156	15-15-15	46	Female	XG
	CIBDL1807137	409	96	1	1	3	1	1+1	5	L:7 R:6	159	15-15-15	52	Female	XG
	CIBDL1807138	427	122	1	L:2 R:1	L:1 R:3	1	1+1	5	7	152	15-15-15	59	Male	XG
	CIBDL1807139	404	104	1	1	3	1	1+1	5	7	151	15-15-15	55	Male	XG
	CIBDL18071311	422	91	1	1	3	1	1+1	5	7	158	15-15-15	47	Female	XG

Note: See Material and Methods section for the abbreviations.

the uncorrected *cyt b* and *cmos* p-distance matrix was compared using MEGA 7.0 (Kumar *et al.*, 2016).

2.3. Morphological analysis Twelve adult snakes (7 males and 5 females) were examined. Information on some morphological characters of known species were obtained from literature (Boie, 1827; Boulenger, 1896, 1900, 1906; Bourret, 1935; Cantor, 1839; Das, 2010; David and Vogel, 2010; de Lang and Vogel, 2006; Doria *et al.*, 2013; Duméril *et al.*, 1854; Günther, 1858, 1864; Leviton, 1970; Smith, 1943; Stuebing and Lian, 2002; Tweedie, 1953; Zhao, 1997; Zhao *et al.*, 1998; Zhao and Adler, 1993; Zhao and Jiang, 1981) and are shown in Table 2. Color description was done according to wiki color-coding.

Terminology for morphological measurements and descriptions is as follows: body and tail length were measured with a tape ruler to the nearest 1 mm; total length (TL), from the tip of snout to the tip of tail; snout-vent length (SVL), from the tip of snout to posterior margin of cloaca; tail length (TaL), from posterior margin of cloaca to the tip of tail. Other measurements were conducted with a digital caliper to the nearest 0.1 mm: head length (HL), from the snout tip to the posterior margin of the mandible; head width (HW) was measured at the widest part of the head on posterior side; the eye horizontal diameter (ED), the greatest diameter of the orbit. Biometric measures were performed exclusively on the right eye. Ratio of tail length to total length (TaL/TL) was recorded. The dorsal scale rows (DSR) were counted at one head length behind head, at mid-body, and at one head length before vent; the number of ventral scales (VEN) was counted according to the method proposed by Dowling (Dowling, 1951), half ventral was counted as one. For subcaudals (SC), first scale under the tail meeting its opposite was regarded as the first subcaudal scale, and the unpaired terminal scute was not included in the number of subcaudals. Paired scales on head were counted on both sides of the head and presented in left/right order.

Table 2 Significant characters of *R. chiwen* sp. nov. and the other known 27 species of the genus *Rhabdophis*.

Species	DSR	Outer dorsal scale row	VEN	SC	PRO	PTO	MT▲
R. chiwen sp. nov.	15–15–15	smooth	151–159	45–59	1–2	2–3	no data
R. pentasupralabialis	15–15–15	smooth	135–162	43–64	1	2–3	18
R. adleri	19–19–17	feebly keeled	150–164	73–88	1–2	3–4	27(25+2)
R. akraios	19–19–17	keeled	167–184	44–66	1	3	19(17+2)
R. angeli	16(15)–15–15	smooth	117–126	39–46	1	3	22–23
R. auriculata	17–17–15	strongly keeled	157–162	76–91	1–2	3	27–32
R. barbouri	19 at midbody	strongly keeled	148–166	96–101	2	3	no data
R. callichroma	19 at midbody	keeled	152–159	79–86	1–2	3	27–35
R. callistus	21 at midbody	strongly keeled	156	76	1	4	no data
R. ceylonensis	19 at midbody	strongly keeled	137–143	40–54	1	3	24–26(22–24+2)
R. chrysargoides	21 at midbody	strongly keeled	154–161	64–79	1	3	24(22+2)
R. chrysargos	19 at midbody	strongly keeled	143–175	60–93	1–2	3	27–35
R. conspicillatus	19 at midbody	keeled or smooth	138–147	40–53	1	3	no data
R. flaviceps	19 at midbody	keeled	120–138	49–60	1	3–4	22–23
R. guangdongensis	15–15–15	smooth	126	39	1	2	20
R. himalayanus	19–19–17	feebly keeled	165–171	82–88	2	3	26(24+2)
R. leonardi	18(17)–17–15	smooth	149–159	43–62	1	2–3	19(17+2)
R. lineatus	19 at midbody	strongly keeled	132–142	66–71	2	3	18(16+2)
R. murudensis	19–19–15(17)	feebly keeled	176–185	63–97	1	3	23(21+2)
R. nigrocinctus	19–19–17	keeled or smooth	150–170	80–97	1	3–4	28(26+2)
R. nuchalis	15–15–15	smooth	144–169	35–65	1	3	18–22
R. plumbicolor	23–27 at midbody	strongly keeled	144–160	27–45	2	3–4	no data
R. rhodomelas	19 at midbody	strongly keeled	126–136	41–56	1–2	3–4	no data
R. rudis	23–23–19	strongly keeled	123–155	44–60	3	3–4	13–20(11–18+2)
R. spilogaster	19 at midbody	feebly keeled	148–156	75–92	1–2	3–4	15
R. subminiatus	19–19–17	keeled or smooth	144–184	56–97	1	3–4	23–26(21–24+2)
R. swinhonis	15–15–15	weakly keeled or smooth	124–165	44–74	1	2–3	19–23(17–21+2–3)
R. tigrinus	19–19–17(15)	keeled or smooth	144–188	38–74	1–2	2–4	22–23(20–21+2)

Notes: See Material and methods section for the data sources.
▲ : total number of maxillary teeth (the number of maxillary teeth before the diastema + the number of maxillary teeth behind the diastema).

Supralabials (SL) were considered being those shields that were behind the rostral and bordering the mouth gap; infralabials (IL) were considered being those shields that were behind the mental, completely below a supralabial and bordering the mouth gap. Number of supraocular (SPO), preoculars (PRO) and postoculars (PTO) were counted as shields above, at the anterior and posterior margin of the orbit. Loreal scales (LR) were counted as scales between the nasal scale and preocular. Number of temporal scales (TEM) were counted as scales behind the postoculars and between the parietal scales and supralabials. The sex was determined by inspection of the existence of hemipenes.

This new species is most similar to and has long been considered as *R. pentasupralabialis*. To illuminate this problem, 22 specimens (11 males and 11 females) collected from the type locality (Jiulong County, JL) of *R. pentasupralabialis* were examined and measured. Morphological characters of specimens were analyzed using Principal Component Analysis (PCA) (Wüster *et al*., 1992). Male specimens were from Jiulong County, Jiguan Mountain and Xingou Village, using the characters VEN, SC, left IL, right IL, left PRO, left PTO and right PTO. Female specimens were from Jiulong County and Xingou Village, using the characters VEN, SC, left IL, right IL, left PTO and right PTO. All statistical analysis were conducted with SPSS 25.0 (IBM Inc., Armonk, U.S.A.).

3. Results

3.1. Molecular phylogenetic analysis We obtained alignments of the mitochondrial gene *cyt b* (1074 bp) and nuclear gene *cmos* (535 bp). Sequence data were uploaded to GenBank, available accession numbers showed in supplementary Table S1. The best evolution models of each partition combination are shown in Table 3. The result show that *cyt b* and *cmos* genes consistent phylogenetic trees were achieved by using Bayesian Inference (BI) and Maximum likelihood RaxML (Figure 1).

The topological structures of combined *cyt b* and *cmos* sequences phylogenetic tree are identical with the earlier study (Takeuchi *et al*., 2018). Our results also strongly supported the monophyly of *Rhabdophis*. The individuals from the Jiguan Mountain and Xingou Village were placed in the genus *Rhabdophis* with a high support (Figure 1). The Results also showed that they share a common ancestor with *R. nuchalis*, *R. leonardi*, and *R. pentasupralabialis*.

The specimen (HKV36838) from Hongya County, Sichuan, China was also nested within the clade with individuals from Jiguan Mountain and Xingou Village.

The uncorrected *p*-distances between species are shown in Table 4. The pairwise distances between species in the genus were wide, ranging from 0.004 to 0.198. The largest disparity occurred between *R. subminiatus* from Thailand and *R. chrysargos* from Malaysia (0.198). In comparison, the specimen of *Rhabdophis* from Jiguan Mountain (CIB116092) differed from *R. pentasupralabialis* from Jiulong County, Sichuan, China (GP1065, type locality) by 0.081, and was different from *R. guangdongensis* (SYSr000018) by 0.066 substitutions per site to the other species. Moreover, the specimen (HKV36838) from Hongya County, Sichuan, China was identified as *R. nuchalis* before (Alfaro and Arnold, 2001), but its pairwise distance with *R. nuchalis* (HT0854 and CIBDL1807208) were rather large, by 0.063 and 0.058 respectively.

Table 3 The best evolution models of each partition combination.

Subset	Best Model	Site (bp)	Subset Partition
1	HKY+I+G	358	*cyt b* 1st
2	HKY+I+G	554	*cmos* 3rd, *cyt b* 2nd
3	GTR+G	358	*cyt b* 3rd
4	K80+I	392	*cmos* 1st, *cmos* 2nd

3.2. Taxonomic conclusions The individuals from the Jiguan Mountain and Xingou Village were placed as monophyletic clade with strong supports (100% in PP and BP). In addition, the *p*-distance between the individuals of the clade and other species indicated very large values (more than 0.058). Combined with further evidence from morphology mentioned below, the individuals from southwestern Sichuan Province represent an undescribed species of the genus *Rhabdophis*. Here, we described it as a new species.

Taxonomy

Rhabdophis chiwen **sp. nov.** Chen, Ding, Chen and Piao, 2019 (Figures 2, 3, 4, 6);

Rhabdophis nuchalis pentasupralabialis: Jiang and Zhao, 1983 pp. 59–62;

Rhabdophis pentasupralabialis: Zhao, 1998, pp. 271–274; Zhao, 2006, pp.268–269; Takeuchi *et al*., 2018, Figure 2 and 3, p. 10226 (part).

Diagnosis. 1) nuchal groove present, with enlarged and paired scales on each side; 2) Dorsal body saddlebrown, DSR in 15 rows throughout, feebly keeled, the outer 1–2 rows smooth; 3) dorsal scales typically with black margins forming some spots and stripes, the margin of the outer row forming two faint dorsolateral black cross-bars alongside body; 4) a black oblique stripe below the eye, often with a black spot between the 2nd and 3rd SL and a black stripe (or separated as black spots) on the 5th SL and the 1st TEM; 5) Eyes dark khaki, pupils black; 6) SL 5, the 3rd and 4th touching the eye: 7) usually 7 ILs (occasionally 6 or 8), the first four contact with anterior chin-shields; 8) 1 LR, and TEM 1+1; 9) VEN 151-159, the outer margin of ventral scales and several lateral rows of dorsal scales forming ventrolateral longitudinal brownish-red coloration, with faint black spots in

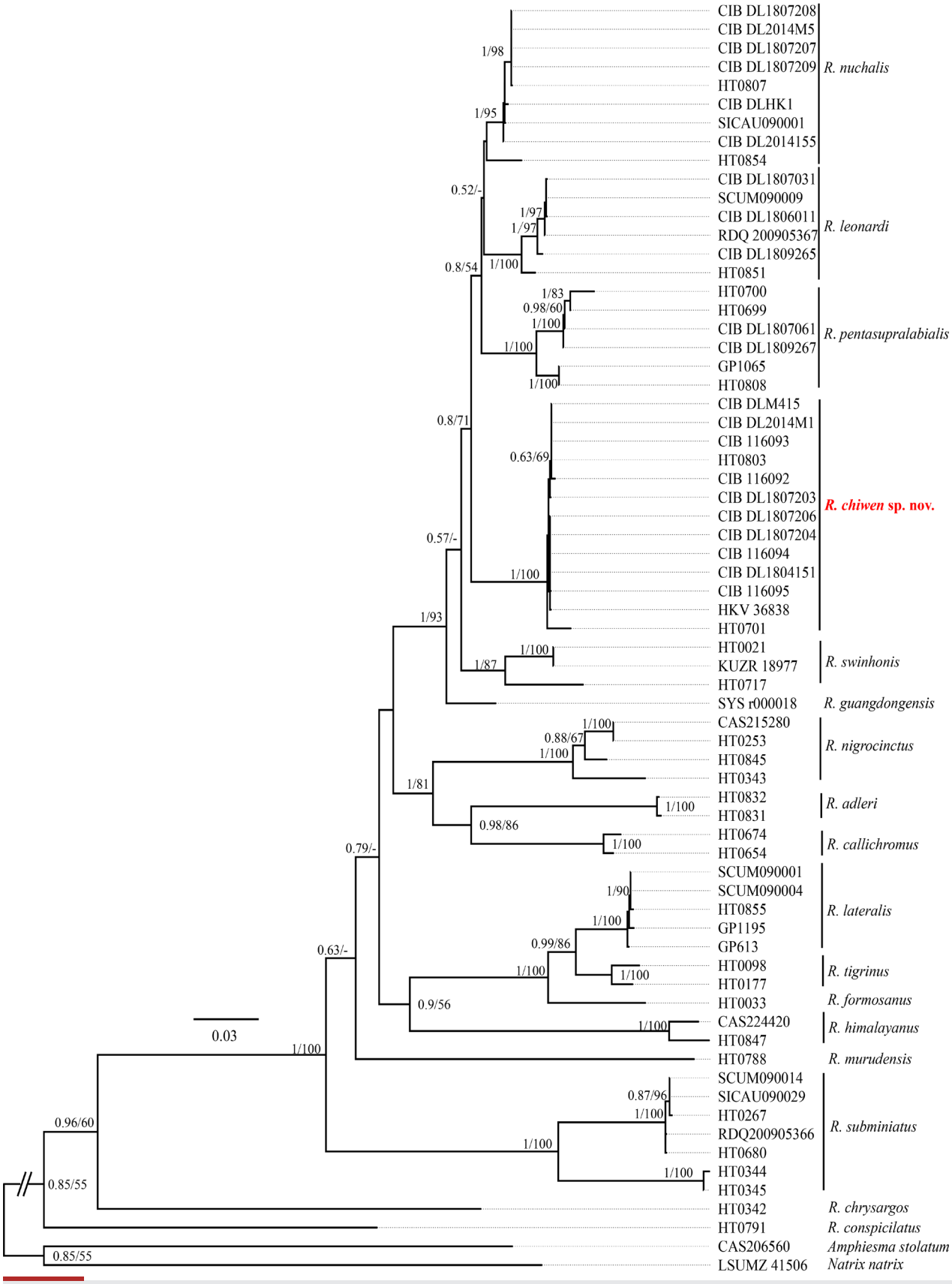

Figure 1 Phylogenetic tree derived from the combined gene fragment (*cmos* + *cyt b*). Bayesian posterior probabilities (PP) and bootstrap probabilities (BP) from maximum likelihood are shown at the nodes.

Table 4 Uncorrected *p*-distances between *Rhabdophis* species based on 1074 base pairs from the mitochondrial genes *cyt b*. Specimens of *R. chiwen* **sp. nov.** are in bold font. ID = Sample ID in Table S1.

ID	Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
63	*R. nuchalis*																							
22	*R. nuchalis*	0.034																						
15	*R. leonardi*	0.045	0.047																					
21	*R. leonardi*	0.044	0.047	0.021																				
13	*R. adleri*	0.053	0.044	0.029	0.038																			
60	*R. pentasupralabialis*	0.055	0.061	0.070	0.066	0.075																		
16	*R. pentasupralabialis*	0.056	0.060	0.070	0.068	0.073	0.031																	
48	*R. chiwen* **sp. nov.**	**0.058**	**0.063**	**0.062**	**0.064**	**0.073**	**0.079**	**0.079**																
69	*R. chiwen* **sp. nov.**	**0.059**	**0.064**	**0.062**	**0.063**	**0.074**	**0.080**	**0.081**	**0.004**															
12	*R. guangdongensis*	0.047	0.053	0.057	0.055	0.058	0.074	0.071	**0.065**	**0.066**														
24	*R. swinhonis*	0.064	0.072	0.073	0.070	0.084	0.091	0.089	**0.086**	**0.085**	0.078													
40	*R. swinhonis*	0.076	0.082	0.086	0.084	0.096	0.097	0.090	**0.086**	**0.086**	0.089	0.066												
47	*R. adleri*	0.132	0.126	0.131	0.132	0.131	0.134	0.128	**0.130**	**0.132**	0.117	0.131	0.136											
34	*R. callichromus*	0.124	0.119	0.127	0.122	0.131	0.130	0.130	**0.121**	**0.121**	0.119	0.123	0.133	0.122										
18	*R. nigrocinctus*	0.120	0.120	0.124	0.130	0.096	0.129	0.125	**0.127**	**0.128**	0.113	0.128	0.129	0.135	0.140									
31	*R. nigrocinctus*	0.135	0.138	0.141	0.138	0.115	0.145	0.143	**0.140**	**0.140**	0.134	0.141	0.141	0.149	0.149	0.050								
6	*R. lateralis*	0.140	0.133	0.144	0.140	0.129	0.146	0.142	**0.137**	**0.140**	0.126	0.144	0.140	0.152	0.151	0.140	0.150							
26	*R. tigrinus*	0.131	0.128	0.138	0.136	0.129	0.142	0.137	**0.127**	**0.130**	0.123	0.153	0.151	0.158	0.152	0.146	0.156	0.065						
25	*R. formosanus*	0.135	0.134	0.140	0.138	0.129	0.141	0.140	**0.141**	**0.139**	0.128	0.141	0.148	0.168	0.160	0.149	0.161	0.090	0.088					
11	*R. himalayanus*	0.148	0.125	0.144	0.134	0.132	0.145	0.145	**0.143**	**0.147**	0.135	0.130	0.140	0.152	0.148	0.152	0.157	0.153	0.157	0.151				
29	*R. subminiatus*	0.152	0.147	0.150	0.153	0.150	0.156	0.156	**0.159**	**0.162**	0.149	0.158	0.156	0.169	0.170	0.166	0.177	0.172	0.165	0.170	0.155			
32	*R. subminiatus*	0.158	0.163	0.149	0.153	0.156	0.165	0.166	**0.157**	**0.158**	0.155	0.151	0.159	0.171	0.165	0.172	0.178	0.178	0.184	0.180	0.169	0.111		
30	*R. chrysargos*	0.190	0.187	0.184	0.184	0.177	0.189	0.188	**0.181**	**0.182**	0.179	0.179	0.178	0.194	0.197	0.191	0.197	0.188	0.182	0.196	0.176	0.197	0.198	
42	*R. conspicilatus*	0.171	0.167	0.167	0.169	0.160	0.168	0.169	**0.166**	**0.168**	0.176	0.165	0.169	0.186	0.181	0.173	0.182	0.179	0.181	0.179	0.181	0.186	0.189	0.166

the middle of each ventral scale and between scales which line a black stripe; 10) anal divided; SC 45–59; 11) PRO 1 (rarely 2) and PTO usually 3 (occasionally 2); 12) medium-sized body (SVL of adult males: 404–431 mm and adult females: 409–476 mm); 13) tail moderate and longer in males than in females (adult males: 104-131 mm, adult females: 91–107 mm).

Etymology. The species name of the new species "*chiwen*" is in reference to the ninth son of Loong in ancient Chinese myth who likes eating fire, and indicates the firefly-eating habit of this new species (Yoshida *et al*., 2020). Its common name is suggested as "Chiwen Keelback" in English and " 螭吻颈槽蛇 " in Chinese.

Holotype. CIB116092, an adult male, collected from Jiguan Mountain, Chongzhou City, Sichuan Province (30°45'57.64" N, 103°14'3.52" E, and 1846 m a.s.l.) collected on 1st July 2019 by Li Ding (Figure 2 and 3).

Paratypes. CIB116093, an adult male, collected from the same locality as **holotype** on 1st July 2019 by Li Ding. CIB116094-95, two specimens, an adult male and an adult female, collected from Xingou Village, Tianquan County, Sichuan Province (29°55'42.39" N, 102°23'9.19" E, and 1461 m a.s.l.) collected on 22nd July 2018 by Li Ding and Zening Chen.

3.3. Description of the holotype Adult male with TL 540 mm (SVL 422 mm and TaL 118 mm). TaL/TL 21.9%. Body elongated and cylindrical. Nuchal groove present; seven scales on each side of groove more or less distinctly enlarged and paired. DSR in 15 rows throughout, feebly keeled, the outer 2 rows smooth. VEN 152; anal divided; SC 56, paired, final spine present at tip of tail.

Head oval and distinctly wider than neck, HL 13.67 mm, 2.53% of TL; HW 9.10 mm, HL/HW 1.50. Eyes are relatively large, with rounded pupil, EW 2.95 mm, EW/HL 21.58%. Rostral semi-circular when viewed from the front, wider than long, barely visible from above. Nostrils large and open laterally, nasal completely divided, in contact with rostral. Internasals slightly shorter than prefrontals. Prefrontals wide, bending to the loreal region. Frontal slightly pentagonal, longer than wide, equal to the distance from the rostral to frontal. LR 1, longitudinally longer. SPO 1; PRO 1; PTO left 2 and right 3; subocular absent. SL 5, the 3rd and 4th touching the eye, with a black oblique stripe below the eye, a black spot between the 2nd and 3rd SL and a black stripe on the 5th SL and the 1st TEM. Mental triangular, width approximately twice of length. IL left 7 and right 6, the first one in contact with each other behind the mental and the first four contact with anterior chin-shields, the 4th to 6th infralabials contact with posterior chin-shields. At the left side, the 7th infralabial slightly longer than the 6th infralabial, but much narrower, width about 1/3 of its height. TEM 2 each side, anterior temporal single and posterior temporal single.

Figure 2 Dorsal (A) and ventral (B) view of the holotype of *Rhabdophis chiwen* **sp. nov.** (CIB116092). Photo by Shengchao Shi.

Hemipenis bilobed, extends to the 11th subcaudal, forked at the level of the 9th subcaudal. When wholly everted, the hemipenes have small and hard spines resembling hooks covering the entire organ with the highest density of spines on the tip. The length of each lobe is about 1/4 of the hemipenis. Basal big spine absent, skin shallow cupped. The centripetal sulcus bifurcates at the fork and extends to the tip of per lobe. Lips evident and smooth (Figure 4).

Coloration of specimens in life. Dorsal body saddlebrown with rusty red. Dorsal scales are typically with black margins forming some scattered spots and stripes (for holotype, those dispersed black spots forming a black stripe around the neck, see Figure 3 A, C, E), the margin of the outer row forming two faint dorsolateral black cross-bars alongside body (Figure 2 A; Figure 5 A, C). Coloration of ventral is seashell and the outer margin of ventral scales and several lateral rows of dorsal scales forming ventrolateral longitudinal brownish-red coloration, with faint black spots in the middle of each ventral scale and between scales at the anterior part of body which line a black stripe and merge into black patches covering the whole scale

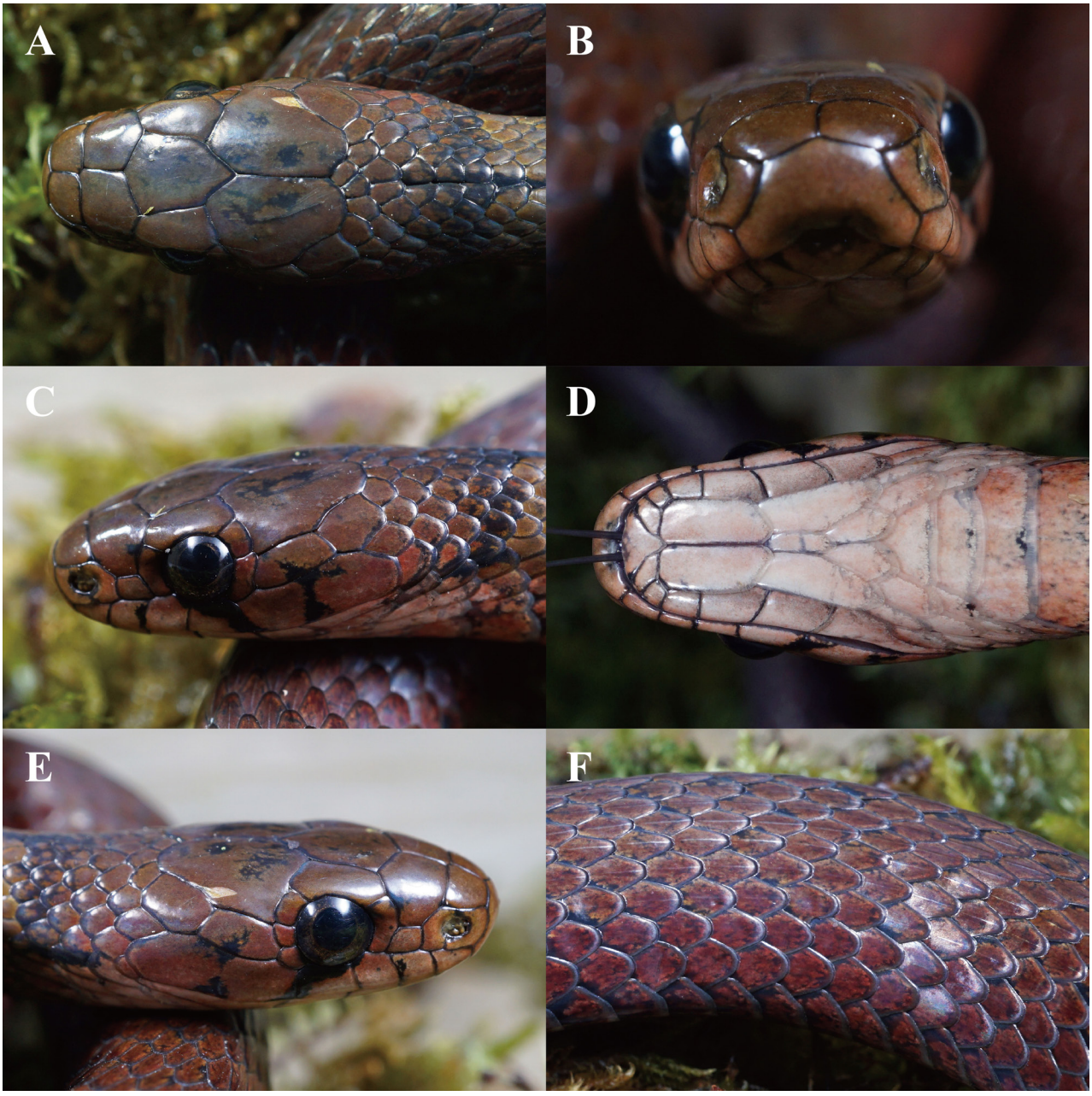

Figure 3 The holotype of *Rhabdophis chiwen* **sp. nov.** (CIB116092) in life. A–E: Different views of head. C and E: showing the same 5 supralabials as *R. pentasupralabialis*. D: ventral view of head, displaying the representative 7 infralabials. F: Lateral view of trunk, demonstrating several smooth outer rows. Photo by Shengchao Shi.

with dispersed brownish-red spots at the posterior part of body (Figure 2 B). Hatchlings similar to adults except for darker dorsal coloration, a yellow stripe present on nape, separated by the nuchal groove (Figure 5 D); ventral scales typically shallow black.

Coloration of specimens in preservative. Coloration pattern in preservative similar to alive, but the faint black spots in the middle of each ventral scale may become much dispersed, forming large black patches alongside the ventral surface after one year in preservative.

3.4 Variation Other specimens generally resemble the holotype except the following characters (Table 1): IL rarely 8. PRO rarely 2 and PTO occasional 2. SVL ranges from 409–476 mm in females and 404–431 mm in males. Female specimens tend to have a shorter tail and fewer SC than male specimens (for TaL 91–107 mm versus 104–131 mm, for SC 45–52 versus 55–59). The ventrolateral longitudinal brownish–red coloration may be lighter (Figure 5 A, C) and faint black spots on the ventral scales could merge into large patches and cover the whole scale at the posterior part of body (Figure 5 B). The dorsal

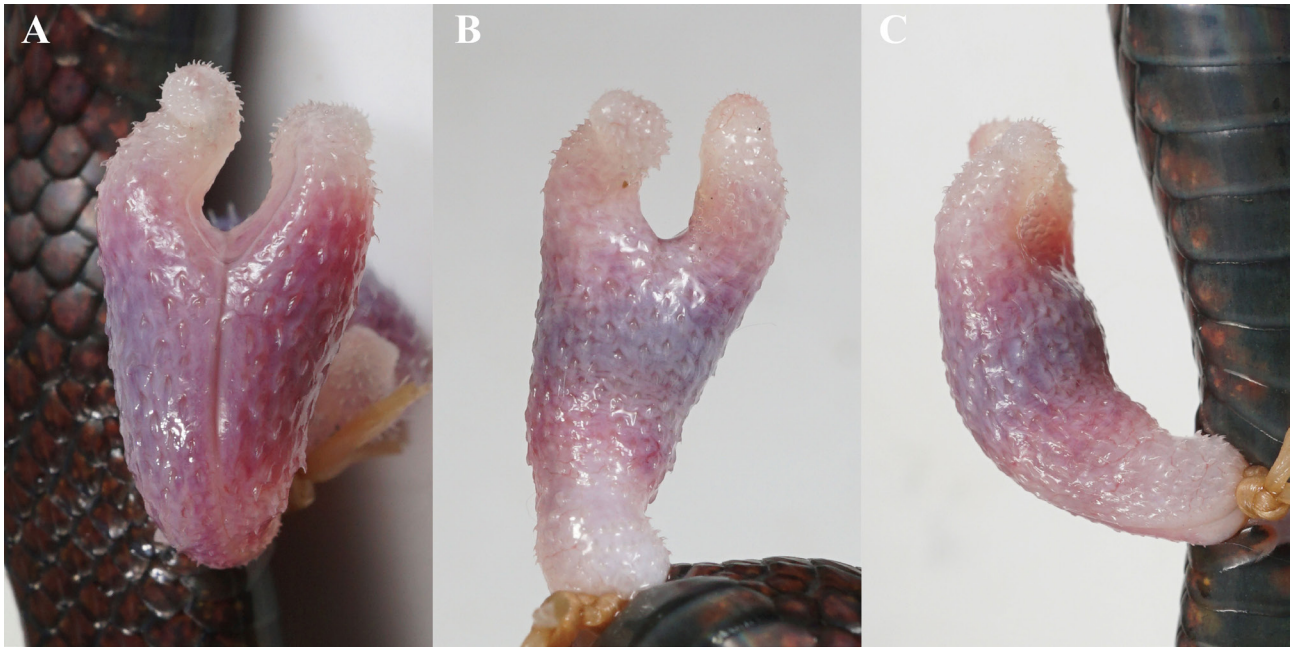

Figure 4 The hemipenis of the holotype of *Rhabdophis chiwen* **sp. nov.** (CIB116092). Photo by Shengchao Shi.

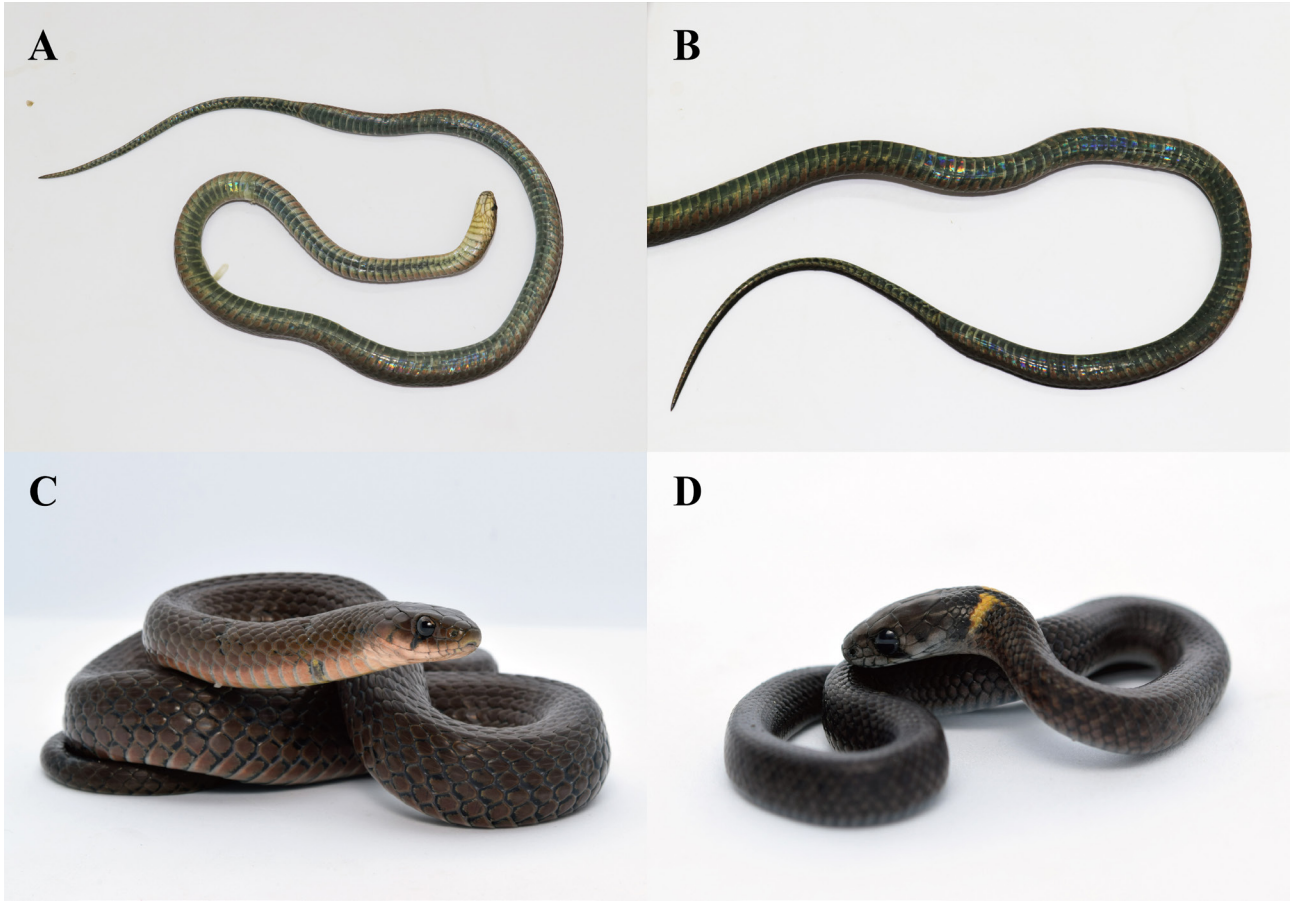

Figure 5 Photos of *Rhabdophis chiwen* **sp. nov.**. A and B: Ventral view of an adult female displaying the coloration variations, photo by Yige Piao. C: An adult female in life. D: A yearling in life. For C and D, photo by Masaya Fukuda.

scales of some specimens (such as CIB116093) are feebly keeled even on the second outer row, with only the outer row smooth.

3.5. Comparisons Comparative data of *R. chiwen* with 27 known species of the genus *Rhabdophis* were obtained from literature (see Materials and methods) and are shown in Table 2.

Currently, 11 species are known to occur in China, and *Rhabdophis chiwen* can be distinguished from these species by following characters (a detailed comparison between *R. chiwen* and *R. pentasupralabialis* is separately listed in a latter paragraph). *R. chiwen* differs from *R. adleri* and *R. himalayanus* by DSR 15 rows throughout vs. DSR 19–19–17 in *R. adleri* and *R. himalayanus*. *R. chiwen* differs from *R. formosanus* and *R. lateralis* by DSR 15 rows throughout vs. DSR 19–19–17(15) in *R. formosanus* and *R. lateralis*. The new species differs from *R. guangdongensis* by 5 SLs, VEN 151–159, SC 45–59, dorsal body saddlebrown vs. 6 SLs, VEN 126, SC 39, brownish–grey coloration in *R. guangdongensis*. The new species is different from *R. leonardi* by DSR 15 rows throughout vs. DSR 18(17)–17–15 in *R. leonardi*. The new species is different from *R. nigrocinctus* and *R. subminiatus* by DSR 15 rows throughout vs. DSR 19–19–17 in *R. nigrocinctus* and *R. subminiatus*. The new species differs from *R. nuchalis* by 5 SLs, 7(6,8) ILs, TEM 1+1, dorsal body saddlebrown vs. 6 SLs, 8(7) ILs, TEM 1+2, olive green coloration with black and magneta spots in *R. nuchalis* (Figure 6). *R. chiwen* differs from *R. swinhonis* by 5 SLs, 7(6) ILs, dorsal body saddlebrown vs. 6 SLs, 8(7) ILs, medium brown coloration with several rows of black spots in *R. swinhonis*.

Rhabdophis chiwen, *R. pentasupralabialis*, *R. guangdongensis*, *R. swinhonis*, *R. angeli* and *R. nuchalis* have the minimal number of dorsal scale rows in this genus, equal to 15 rows. Nevertheless, *R. chiwen* can be readily distinguished from the other five species and all other species of *Rhabdophis* by SLs, DSR, VEN, SC, PRO, PTO or different coloration. Because *R. guangdongensis*, *R. swinhonis* and *R. nuchalis* are distributed in China, their comparisons with *R. chiwen* are listed in the previous paragraph. The new species differs from *R. angeli* by VEN 151–159, SC 45–59, dorsal body saddlebrown vs. VEN 117–126, SC 39–46, brownish coloration with a dorsolateral series of small reddish spots in *R. angeli*.

For the remaining species within the genus *Rhabdophis* except *R. pentasupralabialis*, *R. chiwen* **sp. nov.** can be readily distinguished by its dorsal scales in 15 rows throughout vs. DSR 17–17–15 in *R. auriculata*; DSR 19 at midbody in *R. barbouri*, *R. callichromus*, *R. chrysargos*, *R. conspicillatus*, *R. lineatus* and *R. spilogaster*; DSR 21 at midbody in *R. callistus* and *R. chrysargoides*; DSR 19–19–15(17) in *R. murudensis*; DSR 19–19–17(15) in *R. tigrinus*.

Specimens collected from Xingou Village and Jiguan Mountain differ from specimens of *R. pentasupralabialis* by: 1) larger size (mean TL 536.83 mm) vs. mean TL 483.64 mm in *R. pentasupralabialis* (Table S2); 2) saddlebrown coloration of dorsal body vs. dark green or olive green coloration of dorsal body in *R. pentasupralabialis*; 3) the margin of the outer row forming two faint dorsolateral black cross-bars alongside body vs. absent in *R. pentasupralabialis*; 4) the outer margins of ventral scales and several lateral rows of dorsal scales forming ventrolateral longitudinal brownish-red coloration, with faint black spots in the middle of each ventral scale and between scales which line a black stripe vs. absent in *R. pentasupralabialis* (for the comparison of ventral coloration, see Figure S1).

For male specimens, the first two principal components accounted for 55.900% of cumulative coefficients. Principal component 1 (PC1) accounted for 31.464% and principal component 2 (PC2) for 24.436%. For female specimens, the first two principal components accounted for 64.567%. PC1 accounted for 39.106% and PC2 for 25.461%. Table 5 displays which characters are important in PC1 and PC2 for male and female specimens.

Figure 7 shows the plots of the first two principal components

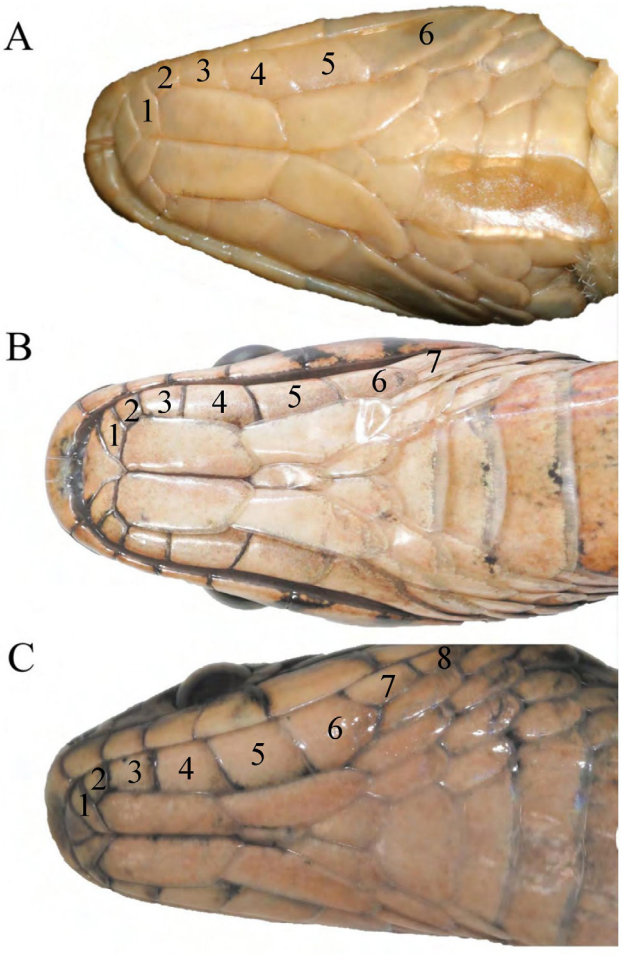

Figure 6 The lateral or ventral view of *R. pentasupralabialis* (A), holotype (CIB116092) of *R. chiwen* **sp. nov.** (B) and *R. nuchalis* (C), displaying the 6 ILs of *R. pentasupralabialis*, 7 ILs of *R. chiwen* **sp. nov.** and 8 ILs of *R. nuchalis*.

Table 5 Component matrix of Principal Component Analysis for male and female specimens.

	Component matrix			
	Males		Females	
	Component		Component	
	1	2	1	2
VEN	−0.759	−0.012	0.697	0.543
SC	−0.351	0.452	−0.452	0.557
left IL	0.647	0.038	0.502	−0.378
right IL	0.781	−0.069	0.487	−0.756
left PRO	0.395	0.821	-	-
left PTO	−0.024	−0.860	0.785	0.139
right PTO	0.563	−0.295	0.742	0.435

Note: See Material and methods section for the abbreviations.

for males (A) and females (B). The PCA for male specimens did not reveal much differentiation among the localities, while the female specimens could be easily distinguished from each other.

3.6. Distribution, habitat and behavior *Rhabdophis chiwen* is currently known to be distributed in several parts of Sichuan Province, including Xingou Village of Tianquan County, Jiguan Mountain of Chongzhou City and Hongya County. During several field surveys in Xingou Village, individuals of this species were commonly encountered, including hatchings, juveniles and adults. At more than six different field sites with the GPS records, we speculate that *Rhabdophis chiwen* lives at the altitude range of 1100-2200m, typically near farmland and the source of water (Figure 8). With the stomach content by forced regurgitation and observation of feeding behaviors in the laboratory, it has been confirmed that this species primarily prey on earthworms and fireflies (Yoshida *et al.*, 2020). The defensive behavior of this species is the typical body lift described by Mori (Mori *et al.*, 2016).

4. Discussion

The genus *Rhabdophis* is widely distributed among Eurasia. Species of this genus are known to occupy a wide range of microhabitat and different altitudes (Zhao, 2006). Sichuan Province lies in the southwestern part of China, which stretches across Palearctic Realm and Oriental Realm, and can mainly be divided into two parts: the western part is the West Sichuan High Plateau and the eastern part is the Sichuan Basin (Zhao, 2002). The complex natural environment provides varied habitats for a large quantity of species (Wang *et al.*, 2010). Currently, 5 species of *Rhabdophis* are reported to inhabit Sichuan Province, i.e. *R. pentasupralabialis*, *R. nuchalis*, *R. leonardi*, *R. subminiatus* and *R. lateralis*. There are doubtful records of *R. nigrocinctus*, because this species typically inhabits low altitude habitat with high humidity rather than dry-hot valleys as the recorded locality in southwestern Sichuan (Li Ding, personal communication). *R. chiwen* was also found in Hongya County during several field surveys, where a specimen (HKV36838) was considered as *R. nuchalis* in a previous study (Alfaro and Arnold, 2001). With our long-term field records since 2012, it is shown that the distribution range of *R. chiwen* is partly overlapped with that of *R. pentasupralabialis* and *R. nuchalis*. Together with *R. leonardi*, these four species all primarily feed on earthworms and form a earthworm-eating clade while most species in this genus prey on frogs and fish (Zhao, 2006; Yoshida *et al.*, 2020). Intriguingly, all these earthworm-eating species can be found in Sichuan, and it remains to be investigated whether and how they show some niche differentiations.

A previous study has pointed out substantial genetic divergence within *R. nigrocinctus*, *R. swinhonis*, *R. nuchalis*, and especially *R. subminiatus*, and suggested the possibility for several undescribed species (Takeuchi *et al.*, 2018). In the study of this genus, our field records clearly support this possibility (Li

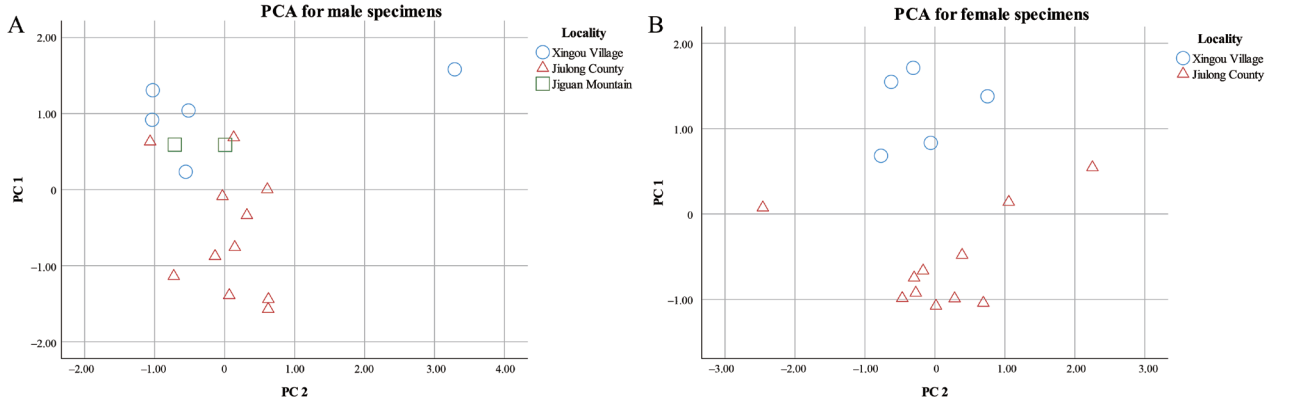

Figure 7 The plots of the first two principal components for males (A) and females (B) of specimens.

Figure 8 Habitat of *Rhabdophis chiwen* in Xingou Village, Tianquan County, Ya'an City, Sichuan Province. Photo by Yige Piao on June 2nd, 2019.

Ding, personal communication). It is sure that the western and southern parts of Sichuan Province still provide shelters for certain cryptic species of *Rhabdophis*.

Acknowledgements This study was supported by the Biodiversity Survey, Observation and Assessment Programme (2019–2023) of Ministry of Ecology and Environment of China to Li DING and Yanqing WU; grants of the National Natural Science Foundation of China (No. 31301882, No. 31970423) to Qin Chen, and Science and Technology Foundation of Sichuan (No.2018SZ0335) to Qin CHEN.; grants from Japan-China Joint Research Project (2014–2016) between the Japan Society for the Promotion of Science (JSPS) and National Natural Science Foundation of China (NSFC, 31411140033) to Yezhong TANG and Akira MORI, and grants from JSPS KAKENHI Grant Numbers JP26440213, JP17H03719, and JP18KK0205 to Akira MORI. In addition, we are much indebted to the Museums of Herpetology in Chengdu Institute of Biology and their staff for their help and permission to examine preserved specimens under their care.

References

Alfaro M. E., Arnold S. J. 2001. Molecular Systematics and Evolution of Regina and the Thamnophiine Snakes. Mol Phylogenet Evol, 21(3): 408–423

Boie F. 1827. [Bemerkungen über Merrem's Versuch eines Systems der Amphibien. Lieferung: Ophidier.] Isis van Oken, 20: 508–566

Boulenger G. A. 1896. Catalogue of the Snakes in the British Museum (Natural History) (Vol. 3). London, UK: Order of the Trustees

Boulenger G. A. 1900. On the reptiles, batrachians (and fishes) collected by the late Mr. John Whitehead in the interior of Hainan. Proc Zool Soc London, 1899: 956–959

Boulenger G. A. 1906. Descriptions of new reptiles from Yunnan. Ann Mag Nat Hist, 17(102): 567–568

Bourret R. E. 1935. Notes herpétologiques sur l'Indochine française. X. Les serpents de la station d'altitude du Tam-dao. Bull. Gen. l'Instr. Publ. Indochine Hanoi, 8: 1–13

Cantor T. E. 1839. Spicilegium serpentium indicorum [part 1]. Proc Zool Soc London, 1839: 31–34

Indraneil D. 2010. A field guide to the reptiles of Thailand, south-east Asia. London, UK: New Holland Publisher

David P., Vogel G. 2010. A new species of the natricine snake genus *Amphiesma* from Borneo (Squamata: Natricidae). Russ J Herpetol, 17(2): 121–127

de Lang R., Vogel G. 2005. The Snakes of Sulawesi: A Field Guide to the Land Snakes of Sulawesi. Frankfurt, Germany: Frankfurt am Main. Edition Chimaira

Doria G., Massimo P., Adriana B., MiCaela T., Erika P. 2013. *Rhabdophis* in the Museum of Genova with Description and Molecular Analysis of A New Species from Sumatra (Reptilia, Serpentes, Colubridae, Natricinae). Annali del Museo Civico di storia naturale, 105: 139–153

Dowling H. G. 1951. A proposed standard system of counting ventrals in snakes. Brit J Herpetol, 1: 97–99

Duméril A. M. C., Bibron G., Duméril A. H. A. 1854. Erpétologie générale ou Histoire Naturelle complète des Reptiles. (Vol. 7). Paris, France: ome septième

Günther A. C. L. G. 1858. Catalogue of colubrine snakes in the collection of the British Museum (Vol. 4). London, UK: Red Lion Court

Günther A. C. L. G. 1864. The Reptiles of British India. London, UK: Ray society

Kumar S., Stecher G., Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol Biol Evol, 33(7), 1870

Lanfear R., Calcott B., Simon Y. W. H., Guindon S. 2012. PartitionFinder: Combined Selection of Partitioning Schemes and Substitution Models for Phylogenetic Analyses. Mol Biol Evol, 29(6): 1695–1701

Leviton A. E. 1970. Description of a New Subspecies of *Rhabdophis Auriculata* in the Philippines: With Comments on the Zoogeography of Mindanao Island. Proc Cali Acad Sci, 38: 347–362

Malnate E. V. 1960. Systematic Division and Evolution of the Colubrid Snake Genus *Natrix*, with Comments on the Subfamily Natricinae. P Acad Nat Sci Phila, 112: 41–71

Mori A., Burghardt G. M., Savitzky A. H., Roberts K. A., Hutchinson D. A., Goris R. C. 2012. Nuchal glands: a novel defensive system in snakes. Chemoecology, 22(3): 187–198

Mori A., Jono T., Takeuchi H., Ding L., Silva A., Mahaulpatha D., Tang Y. Z. 2016. Morphology of the nucho-dorsal glands and related defensive displays in three species of Asian natricine snakes. J. Zool., 300(1): 18–26

Nguyen L. T., Schmidt H. A., von Haeseler A., Minh B. Q. 2015. IQ-TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies. Mol Biol Evol, 32(1): 268–274

Ronquist F., Teslenko M., van der Mark P., Ayres D. L., Darling A., Hohna S., Huelsenbeck J. P. 2012. MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice Across a Large Model Space. Stst Biol, 61(3): 539–542

Smith M. A. 1943. The Fauna of British India Ceylon and Burma including the whole of Indo-Chinese sub-region. London, UK: Taylor and Francis

Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinf, 30(9): 1312–1313

Stuebing R. B., Lian T. F. 2002. Notes on the fire-lipped keelback *Rhabdophis murudensis* (Smith, 1925) (Ophidia: Colubridae: Natricinae) from northern Borneo. Raffles B Zool, 50(1): 227–230

Takeuchi H., Savitzky A. H., Ding L., de Silva A., Das I., Nguyen T. T., Tsai T. S., Jono T., Zhu G. X., Mahaulpatha D., Tang Y. Z., Mori A. 2018. Evolution of nuchal glands, unusual defensive organs of Asian natricine snakes (Serpentes: Colubridae), inferred from a molecular phylogeny. Ecol Evol, 8(20): 10219–10232

Tweedie M. 1953. The snakes of Malaya. Singapore: Authority Government Printing Office

Wang Q. G., Zhao X. H., Zhao Y., Ding F., Xing K. J., Li S. B. 2010. Biodiversity database information system of Sichuan Province (In Chinese). Chin J Ecol, 29(9): 1875–1880

Wüster W., Otsuka S., Malhotra A., Thorpe R. S. 1992. Population systematics of Russell's viper: a multivariate study. Biol J Linn Soc Lond, 47(1): 97–113

Yoshida T., Ujiie R., Savitzky A. H., Jono T., Inoue T., Yoshinaga N., Aburaya S., Aoki W., Takeuchi H., Ding L., Chen Q., Cao C., Tsai T. S., de Silva A., Mahaulpatha D., Nguyen T. T., Tang Y., Mori N., Mori A. 2020. Dramatic dietary shift maintains sequestered toxins in chemically defended snakes. Proc Nat Acad Sci, 117(11): 5964–5969

Zhao E. M. 1997. A new species of *Rhabdophis* (Serpentes: Colubridae) from Hainan Island, China. Asian Herpetol Res, 7: 166–169

Zhao E. M. 2006. Snakes of China (Vol. 279). Hefei, China: Anhui Science and Technology Press (In Chinese)

Zhao E. M., Huang M. H., Zong Y. 1998. Fauna Sinica Reptilia, vol. 3: Squamata: Serpentes (Vol. 522). Beijing, China: Science Press (In Chinese)

Zhao E. M., Adler K. A. 1993. Herpetology of China [Oxford, Ohio]: Contributions to Herpetology 10. Society for the Study of Amphibians and Reptiles

Zhao E. M., Jiang Y. M. 1981. Studies on amphibians and reptiles of Mt. Gongga Shan, Sichuan, China, I. A new species and a new subspecies of snakes from Sichuan (In Chinese). Acta Herpetol Sinica, 1981, 5(7): 53–58

Zhao E. M. 2002. Reptile Fauna and Zoogeographic Division of Sichuan (In Chinese). Sic J Zool, 21(3): 157–160

Zhu G. X., Wang Y. Y., Takeuchi H., Zhao E. M. 2014. A new species of the genus *Rhabdophis* Fitzinger, 1843 (Squamata: Colubridae) from Guangdong Province, southern China. Zootaxa, 3765(5): 469–480

Handling Editor: Chen YANG

How to cite this article:

Piao Y. G., Chen Z. N., Wu Y. Q., Shi S. C., Takeuchi H., Jono T., Fukuda M., Mori A., Tang Y. Z., Chen Q., Ding L. A New Species of the Genus *Rhabdophis* Fitzinger, 1843 (Squamata: Colubridae) in Southwestern Sichuan, China. Asian Herpetol Res, 2020, 11(2): 95–107. DOI: 10.16373/j.cnki.ahr.190068

Appendix

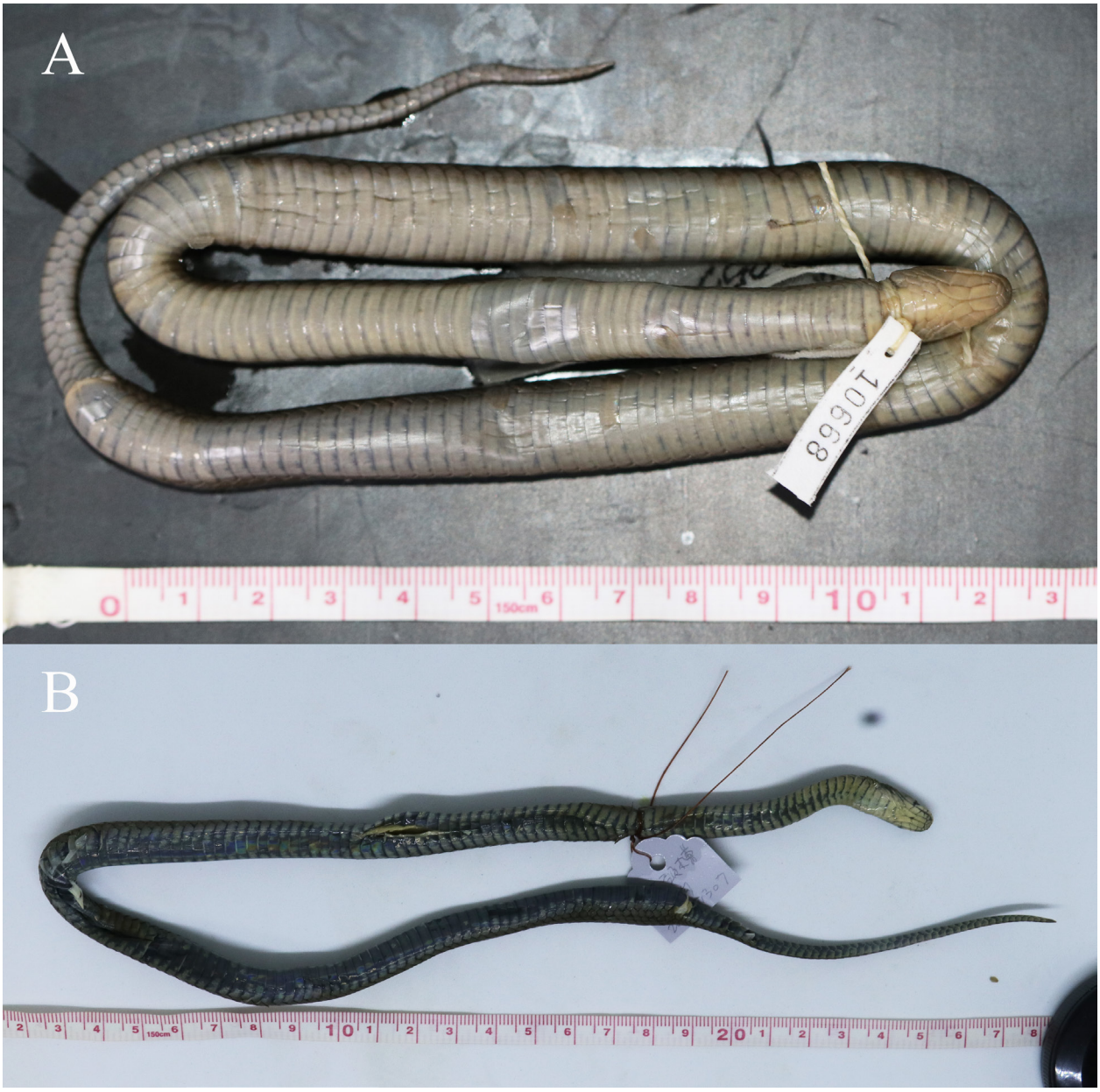

Figure S1 The ventral view of *R. pentasupralabialis* (CIB10668) (A) from type locality and *R. chiwen* **sp. nov.** (CIBDL1807137) (B) from Xingou Village, displaying the black spots and patches on the ventral scales in *R. chiwen* **sp. nov.** vs. absent in *R. pentasupralabialis*. Both specimens were kept in preservative for more than one year.

Table S1 Information of all sequence data used in the study.

Sample ID	Species	Individual No.	Country and Locality	*cyt b*	*cmos*
1	*Amphiesma stolatum*	CAS206560	Bago Div., Myanmar	AF471030	AF471097
2	*Natrix natrix*	LSUMZ41506	Sheppey Island, Kent, United Kingdom	AF471059	AF471121
3	*Rhabdophis swinhonis*	KUZR18977	Taiwan, China	AB842176	AB861888
4	*Rhabdophis lateralis_2*	GP613	China	GQ281785	JQ687444
5	*Rhabdophis tigrinus*	GP1195	Huangshan, Anhui, China	KF765395	KF765390
6	*Rhabdophis tigrinus*	SCUM090004	Tianshui, Gansu, China	KF765396	KF765391
7	*Rhabdophis tigrinus*	SCUM090001	Longnan, Gansu, China	KF765397	KF765392
8	*Rhabdophis subminiatus*	RDQ200905366	Baoshan, Yunnan, China	KF765398	KF765393
9	*Rhabdophis subminiatus*	SCUM090014	Panzhihua, Sichuan, China	KF800927	KF765394
10	*Rhabdophis subminiatus*	SICAU090029	Hong Kong, China	KF800928	KF800918
11	*Rhabdophis himalayanus*	CAS224420	Kachin State, Myanmar	KF800929	KF800919
12	*Rhabdophis guangdongensis*	SYSr000018	Aizhai, Renhuay, Guangdong, China	KF800930	KF800920
13	*Rhabdophis adleri*	WJC20090801	Hainan, China	KF800931	KF800921
14	*Rhabdophis leonardi*	RDQ200905367	Dali, Yunnan, China	KF800932	KF800922
15	*Rhabdophis leonardi*	SCUM090009	Panzhihua, Sichuan, China	KF800933	KF800923
16	*Rhabdophis pentasupralabialis*	GP1065	Jiulong, Sichuan, China	KF800934	KF800924
17	*Rhabdophis nuchalis*	SICAU090001	Shennongjia, Hubei, China	KF800935	KF800925
18	*Rhabdophis nigrocinctus*	CAS215280	Shan State, Myanmar	KF800936	KF800926
19	*Rhabdophis nigrocinctus_3*	HT0845	China	LC325298	
20	*Rhabdophis himalayanus_1*	HT0847	China	LC325299	LC325746
21	*Rhabdophis leonardi_1*	HT0851	China	LC325300	LC325747
22	*Rhabdophis nuchalis_4*	HT0854	China	LC325301	LC325748
23	*Rhabdophis lateralis_1*	HT0855	China	LC325302	
24	*Rhabdophis swinhonis_1*	HT0021	Taiwan, China	LC325303	LC325749
25	*Rhabdophis formosanus_1*	HT0033	Taiwan, China	LC325304	LC325750
26	*Rhabdophis tigrinus_1*	HT0098	Japan	LC325305	LC325751
27	*Rhabdophis tigrinus_2*	HT0177	Japan	LC325306	LC325752
28	*Rhabdophis nigrocinctus_1*	HT0253	Thailand	LC325307	LC325753
29	*Rhabdophis subminiatus_1*	HT0267	Laos	LC325308	LC325754
30	*Rhabdophis chrysargos*	HT0342	Malaysia	LC325313	LC325759
31	*Rhabdophis nigrocinctus_2*	HT0343	Thailand	LC325314	LC325760
32	*Rhabdophis subminiatus_2*	HT0344	Thailand	LC325315	LC325761
33	*Rhabdophis subminiatus_3*	HT0345	Thailand	LC325316	LC325762
34	*Rhabdophis callichromus_1*	HT0654	Vietnam	LC325324	LC325770
35	*Rhabdophis callichromus_2*	HT0674	Vietnam	LC325325	LC325771
36	*Rhabdophis subminiatus_4*	HT0680	Vietnam	LC325328	LC325774
37	*Rhabdophis pentasupralabialis_1*	HT0699	China	LC325331	LC325777
38	*Rhabdophis pentasupralabialis_2*	HT0700	China	LC325332	LC325778
39	*Rhabdophis nuchalis_1*	HT0701	China	LC325333	LC325779
40	*Rhabdophis swinhonis_2*	HT0717	Taiwan,China	LC325334	LC325780
41	*Rhabdophis murudensis*	HT0788	Malaysia	LC325341	LC325787
42	*Rhabdophis conspicilatus*	HT0791	Malaysia	LC325342	LC325788
43	*Rhabdophis nuchalis_2*	HT0803	China	LC325352	LC325798
44	*Rhabdophis nuchalis_3*	HT0807	China	LC325353	LC325799
45	*Rhabdophis pentasupralabialis_3*	HT0808	China	LC325354	LC325800
46	*Rhabdophis adleri_1*	HT0831	China	LC325356	LC325802
47	*Rhabdophis adleri_2*	HT0832	China	LC325357	LC325803
48	*Rhabdophis nuchalis*	HKV36838	Hongya County, Sichuan, China	AF402907	
49	*Rhabdophis chiwen* **sp. nov.**	CIB DL2014155	Jiguan Mountain, Sichuan, China	MN656319	MN656340

(Continued Table S1)

Sample ID	Species	Individual No.	Country and Locality	*cyt b*	*cmos*
50	*Rhabdophis chiwen* **sp. nov.**	CIB DL1807203	Jiguan Mountain, Sichuan, China	MN656320	MN656341
51	*Rhabdophis chiwen* **sp. nov.**	CIB DL1807204	Jiguan Mountain, Sichuan, China	MN656321	MN656342
52	*Rhabdophis chiwen* **sp. nov.**	CIB DL1807206	Jiguan Mountain, Sichuan, China	MN656322	MN656343
53	*Rhabdophis nuchalis*	CIB DLHK1	Chongqing, China	MN656323	MN656344
54	*Rhabdophis chiwen* **sp. nov.**	CIB DL2014M1	Jiguan Mountain, Sichuan, China	MN656324	MN656345
55	*Rhabdophis chiwen* **sp. nov.**	CIB DLM415	Jiguan Mountain, Sichuan, China	MN656325	MN656346
56	*Rhabdophis nuchalis*	CIB DL2014M5	Longnan, Gansu, China	MN656326	MN656347
57	*Rhabdophis leonardi*	CIB DL1806011	Panzhihua, Sichuan, China	MN656327	MN656348
58	*Rhabdophis leonardi*	CIB DL1807031	Panzhihua, Sichuan, China	MN656328	MN656349
59	*Rhabdophis pentasupralabialis*	CIB DL1807061	Panzhihua, Sichuan, China	MN656329	MN656350
60	*Rhabdophis pentasupralabialis*	CIB DL1809267	Panzhihua, Sichuan, China	MN656330	MN656351
61	*Rhabdophis nuchalis*	CIB DL1807207	Tangjiahe, Sichuan, China	MN656333	MN656354
62	*Rhabdophis nuchalis*	CIB DL1807208	Tangjiahe, Sichuan, China	MN656334	MN656355
63	*Rhabdophis nuchalis*	CIB DL1807209	Tangjiahe, Sichuan, China	MN656335	MN656356
64	*Rhabdophis chiwen* **sp. nov.**	CIB116094	Xingou Village, Sichuan, China	MN656336	MN656357
65	*Rhabdophis chiwen* **sp. nov.**	CIB DL1804151	Xingou Village, Sichuan, China	MN656337	MN656358
66	*Rhabdophis chiwen* **sp. nov.**	CIB116095	Xingou Village, Sichuan, China	MN656338	MN656359
67	*Rhabdophis leonardi*	CIB DL1809265	Yingjiang, Yunnan, China	MN656318	MN656339
68	*Rhabdophis chiwen* **sp. nov.**	CIB116092	Jiguan Mountain, Sichuan, China	MN656331	MN656352
69	*Rhabdophis chiwen* **sp. nov.**	CIB116093	Jiguan Mountain, Sichuan, China	MN656332	MN656353

Table S2 Morphological characters of specimens used in the study. See Material and methods section for the abbreviations. For the IL list, the numbers in brackets indicate the number of infralabials touching the anterior chin-shields.

Locality	Number	Sex	SVL	TL	TaL	ED	HL	HW	Ven	Sc	ASR	MSR	DSR	SL-left	S-right	IL-left	IL-right	PRO	PTO	LR	TEM
Xingou Village	CIBDL1807135	Female	476	583	107	2.97	14.55	11.25	156	46	15	15	15	2-2-1	2-2-1	7(4)	7(4)	1	3	1	2
Xingou Village	CIBDL1807132	Male	427	555	128	3.05	13.39	9.22	154	58	15	15	15	2-2-1	2-2-1	8(4)	7(4)	1	3	1	2
Xingou Village	CIB116094	Male	411	524	113	2.95	13.06	9.02	154	55	15	15	15	2-2-1	2-2-1	7(4)	7(4)	1	3	1	2
Xingou Village	CIBDL1807133	Male	409	537	128	2.63	12.95	7.95	157	58	15	15	15	2-2-1	2-2-1	7(4)	7(4)	1	3	1	2
Xingou Village	CIBDL1807138	Male	427	549	122	2.63	13.90	8.45	152	59	15	15	15	2-2-1	2-2-1	7(4)	7(4)	L:2 R:1	L:1 R:3	1	2
Xingou Village	CIBDL18071311	Female	422	513	91	2.86	12.60	8.84	158	47	15	15	15	2-2-1	2-2-1	7(4)	7(4)	1	3	1	2
Xingou Village	CIBDL1807134	Female	411	509	98	2.55	11.91	7.33	159	49	15	15	15	2-2-1	2-2-1	7(4)	6(4)	1	L:3 R:2	1	2
Xingou Village	CIBDL1807139	Male	404	508	104	2.32	13.61	9.02	151	55	15	15	15	2-2-1	2-2-1	7(4)	7(4)	1	3	1	2
Xingou Village	CIB116095	Female	461	557	96	2.70	13.90	8.96	157	45	15	15	15	2-2-1	2-2-1	6(4)	7(4)	1	L:3 R:2	1	2
Xingou Village	CIBDL1807137	Female	409	505	96	2.90	12.13	7.61	159	52	15	15	15	2-2-1	2-2-1	7(4)	6(4)	1	3	1	2
Jiguan Mountain	CIB116092	Male	422	540	118	2.95	13.67	9.10	152	56	15	15	15	2-2-1	2-2-1	7(4)	6(4)	1	L:2 R:3	1	2
Jiguan Mountain	CIB116093	Male	431	562	131	2.88	13.55	9.28	155	57	15	15	15	2-2-1	2-2-1	7(4)	7(4)	1	3	1	2
Jiulong County	CIB10657	Male	382	486	104	2.17	12.08	7.96	158	57	14	15	15	2-2-1	2-2-1	5(4)	6(4)	1	3	1	2
Jiulong County	CIB10660	Female	383	474	91	2.60	13.01	8.12	150	49	15	15	15	2-2-1	2-2-1	6(4)	5(4)	1	L:3 R:2	1	2
Jiulong County	CIB10689	Male	360	454	94	2.38	11.77	8.51	154	56	15	15	15	2-2-1	2-2-1	6(4)	6(4)	1	L:2 R:3	1	2
Jiulong County	CIB10681	Female	419	528	109	2.25	13.60	8.43	160	52	15	15	15	2-2-1	2-2-1	5(4)	5(4)	1	3	1	2
Jiulong County	CIB10655	Male	370	475	105	2.25	11.38	8.06	157	59	15	15	15	2-2-1	2-2-1	7(4)	5(3)	1	L:3 R:2	1	2
Jiulong County	CIB10690	Female	427	535	108	2.37	13.25	8.73	155	55	15	15	15	2-2-1	2-2-1	6(4)	5(4)	1	2	1	2
Jiulong County	CIB10636	Male	367	466	99	2.17	12.88	8,71	156	55	14	15	15	2-2-1	2-2-1	6(4)	6(4)	1	2	1	2
Jiulong County	CIB10650	Female	391	498	107	2.17	12.70	9.30	154	51	14	15	15	2-2-1	2-2-1	6(4)	5(4)	1	2	1	2
Jiulong County	CIB10696	Male	339	425	86	2.03	11.44	7.90	153	55	15	15	15	2-2-1	2-2-1	6(4)	7(4)	1	3	1	2
Jiulong County	CIB10654	Male	348	444	96	2.23	11.83	7.98	152	56	15	15	15	2-2-1	2-2-1	6(4)	6(4)	1	2	1	2
Jiulong County	CIB10684	Female	398	500	102	2.22	12.76	8.82	152	52	14	15	15	2-2-1	2-2-1	6(4)	6(4)	1	2	1	2
Jiulong County	CIB10823	Female	347	452	105	1.96	11.20	7.83	157	62	15	15	15	2-2-1	2-2-1	7(4)	6(4)	1	L:2 R:3	1	2
Jiulong County	CIB10850	Female	390	496	106	2.33	12.95	9.15	153	57	15	15	15	2-2-1	2-2-1	6(4)	6(4)	1	2	1	2
Jiulong County	CIB10800	Female	394	515	121	2.22	13.04	8.69	157	61	15	15	15	2-2-1	2-2-1	7(4)	6(4)	1	2	1	2
Jiulong County	CIB10812	Male	389	501	112	2.17	12.54	8.08	159	58	15	15	15	2-2-1	2-2-1	6(4)	6(4)	1	2	1	2
Jiulong County	CIB10691	Female	382	473	91	2.32	12.99	9.34	152	46	15	15	15	2-2-1	2-2-1	7(4)	8(5)	1	2	1	2
Jiulong County	CIB10668	Female	418	530	112	2.09	13.67	9.21	153	53	15	15	15	2-2-1	2-2-1	6(4)	6(4)	1	2	1	2
Jiulong County	CIB10652	Female	437	548	111	2.16	14.16	9.88	154	51	15	15	15	2-2-1	2-2-1	6(4)	6(4)	1	2	1	2
Jiulong County	CIB10656	Male	366	467	101	2.29	12.05	7.93	155	58	15	15	15	2-2-1	2-2-1	7(4)	7(4)	1	2	1	2
Jiulong County	CIB10659	Male	382	488	106	2.37	12.75	8.39	160	58	15	15	15	2-2-1	2-2-1	6(4)	6(4)	1	2	1	2
Jiulong County	CIB10836	Male	338	432	94	2.21	12.23	7.68	153	60	15	15	15	2-2-1	2-2-1	6(4)	5(4)	1	3	1	2
Jiulong County	CIB10841	Male	347	453	106	1.82	11.85	7.52	152	55	15	15	15	2-2-1	2-2-1	7(4)	7(4)	1	2	1	2